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(12) **United States Patent**  
**Williamson, IV et al.**

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(54) **CASSETTE AND EMBEDDING ASSEMBLY  
FOR HANDLING AND HOLDING TISSUE  
SAMPLES DURING PROCESSING,  
EMBEDDING AND MICROTOME  
PROCEDURES, STAGING DEVICES  
THEREFORE, AND METHODS THEREFOR**

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(51) **Int. Cl.**  
**B01L 9/00** (2006.01)

(52) **U.S. Cl.** ..... **422/104; 422/102; 435/297.5**

(58) **Field of Classification Search** ..... **422/102,**  
**422/104; 435/297.5**

See application file for complete search history.

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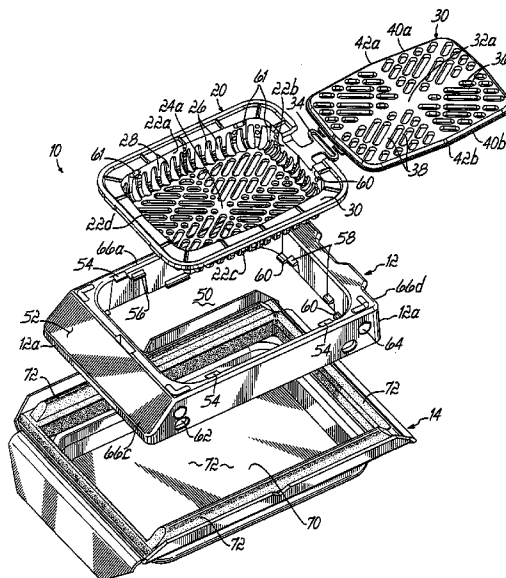
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(57) **ABSTRACT**

The disclosure relates to a cassette, frame and mold for hold-  
ing a tissue sample during an embedding and microtoming  
process, and related methods. The cassette is sectionable in a  
microtome and includes a body with a bottom wall and a  
plurality of side walls. First and second side walls are gener-  
ally V-shaped to present an apex of the “V” to the microtome  
blade. A lid of the cassette is stiffer than the bottom wall of the  
cassette to assist with positioning the tissue sample. The side  
walls of the cassette are perforated so as to significantly  
reduce the amount of cassette material that must be cut by the  
microtome blade. In one embodiment, to additionally reduce  
blade wear the ribs on one side wall are offset lengthwise  
relative to the ribs on an opposite side wall. An upper flange of  
the cassette includes depressions configured to register with  
detents in the frame.

**8 Claims, 8 Drawing Sheets**



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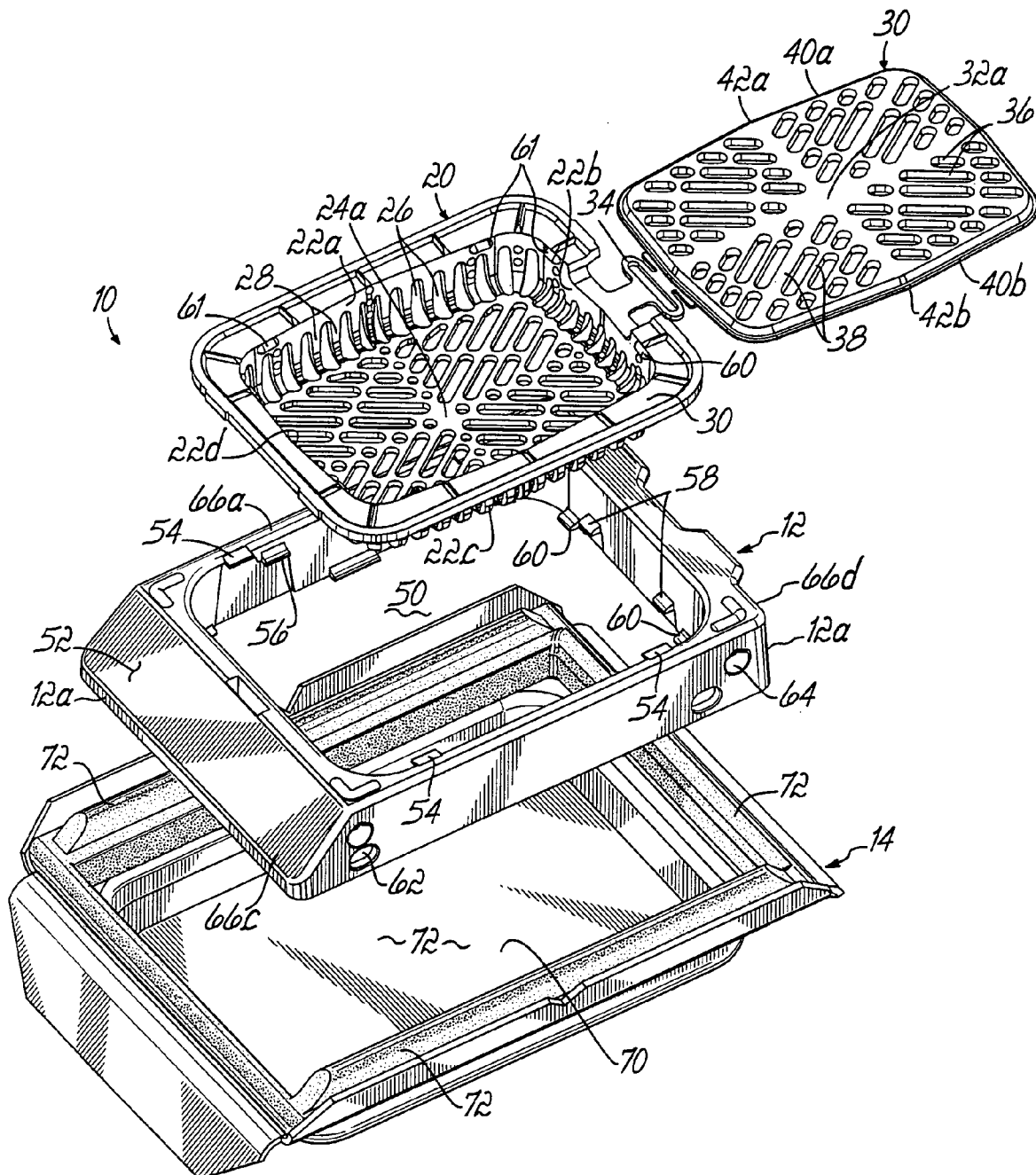


FIG. 1

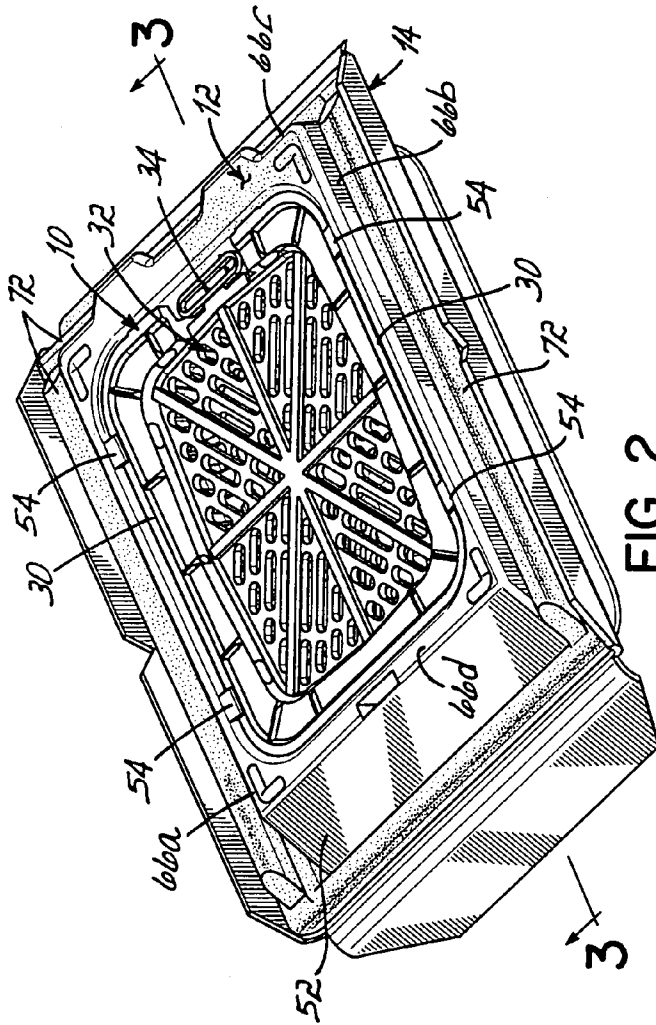


FIG. 2

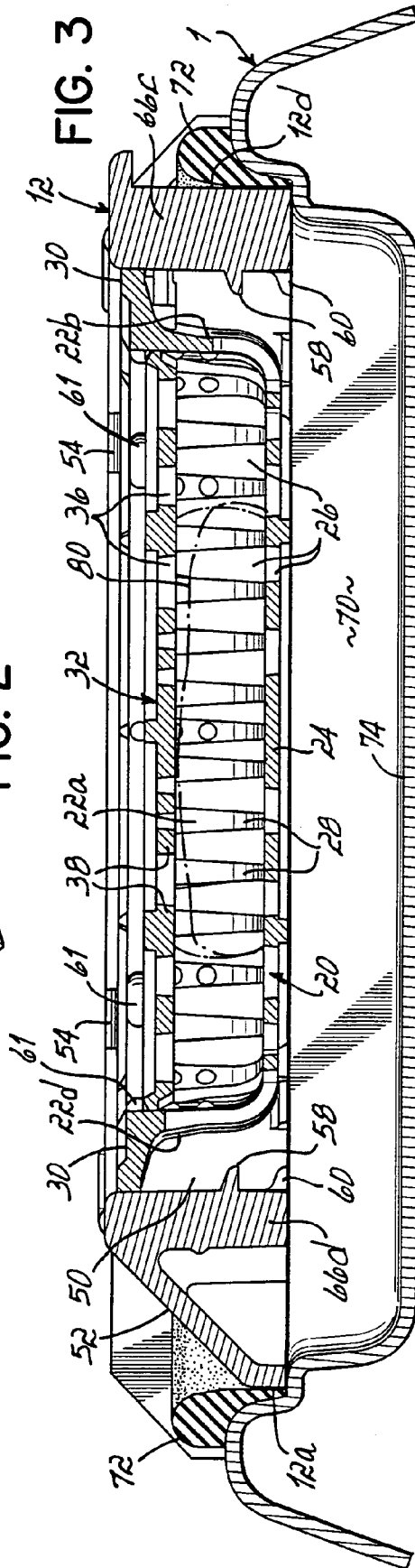
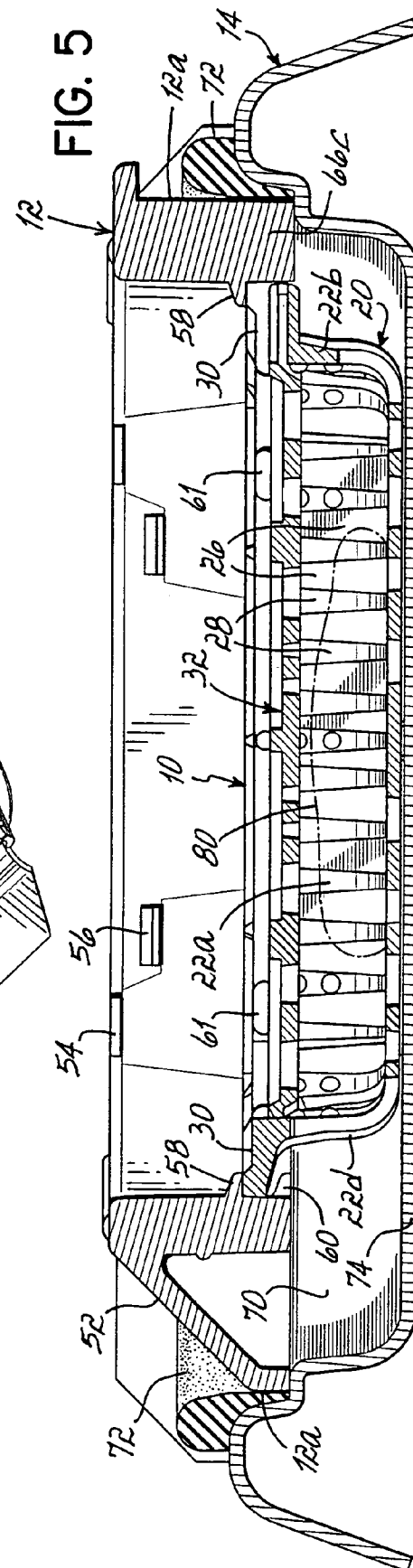
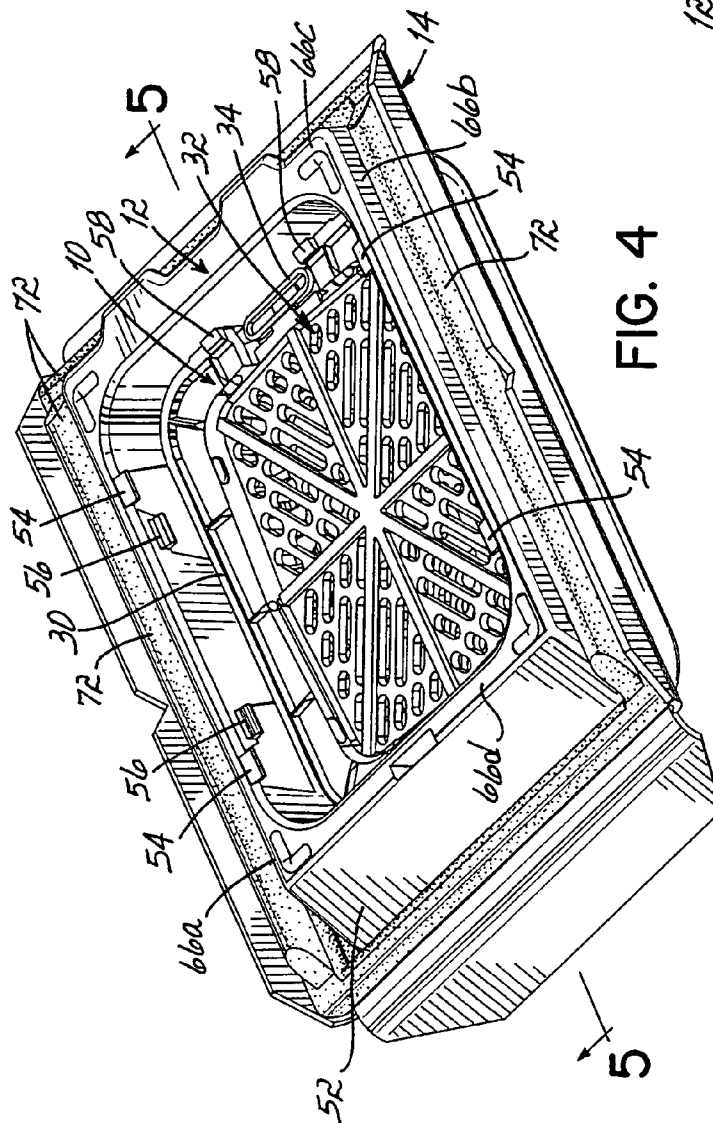


FIG. 3



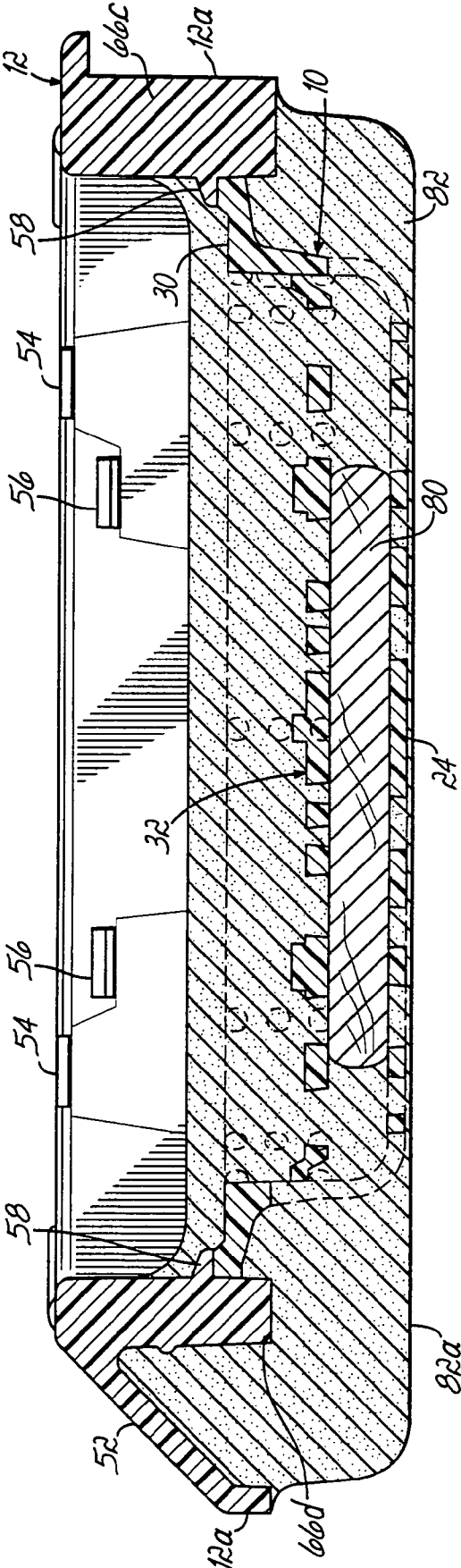


FIG. 6

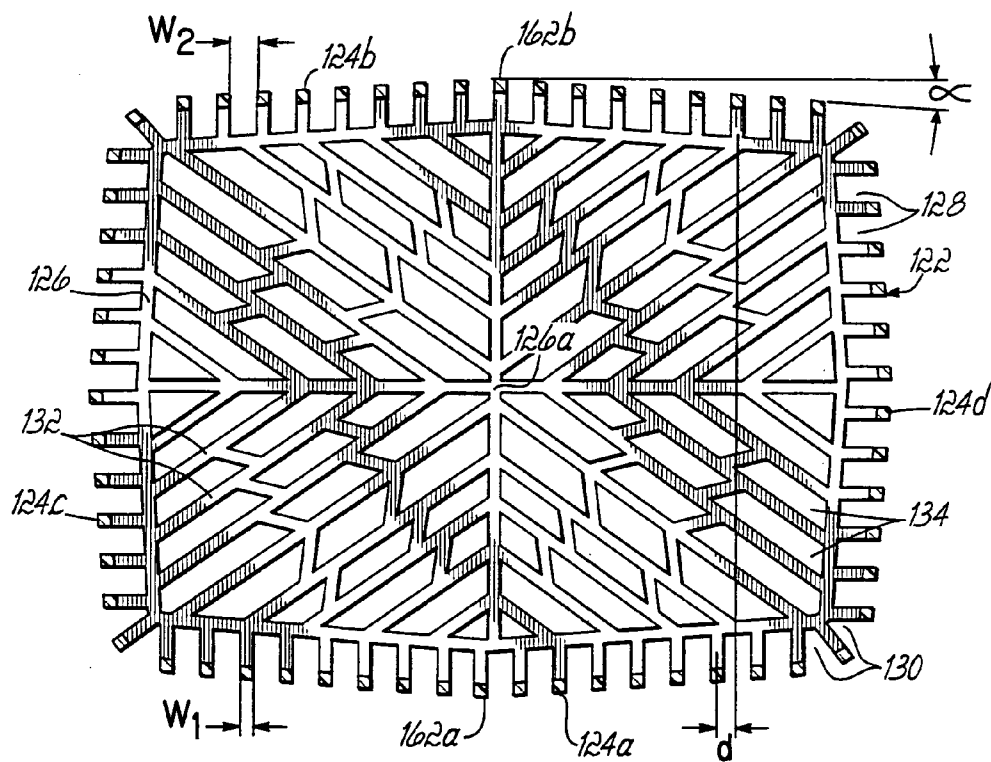
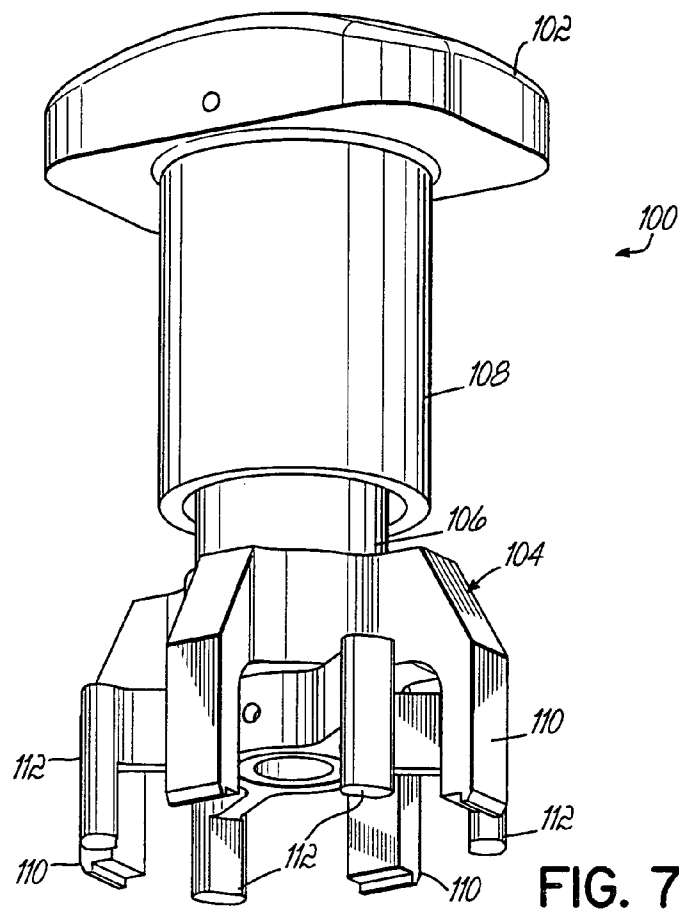


FIG. 8

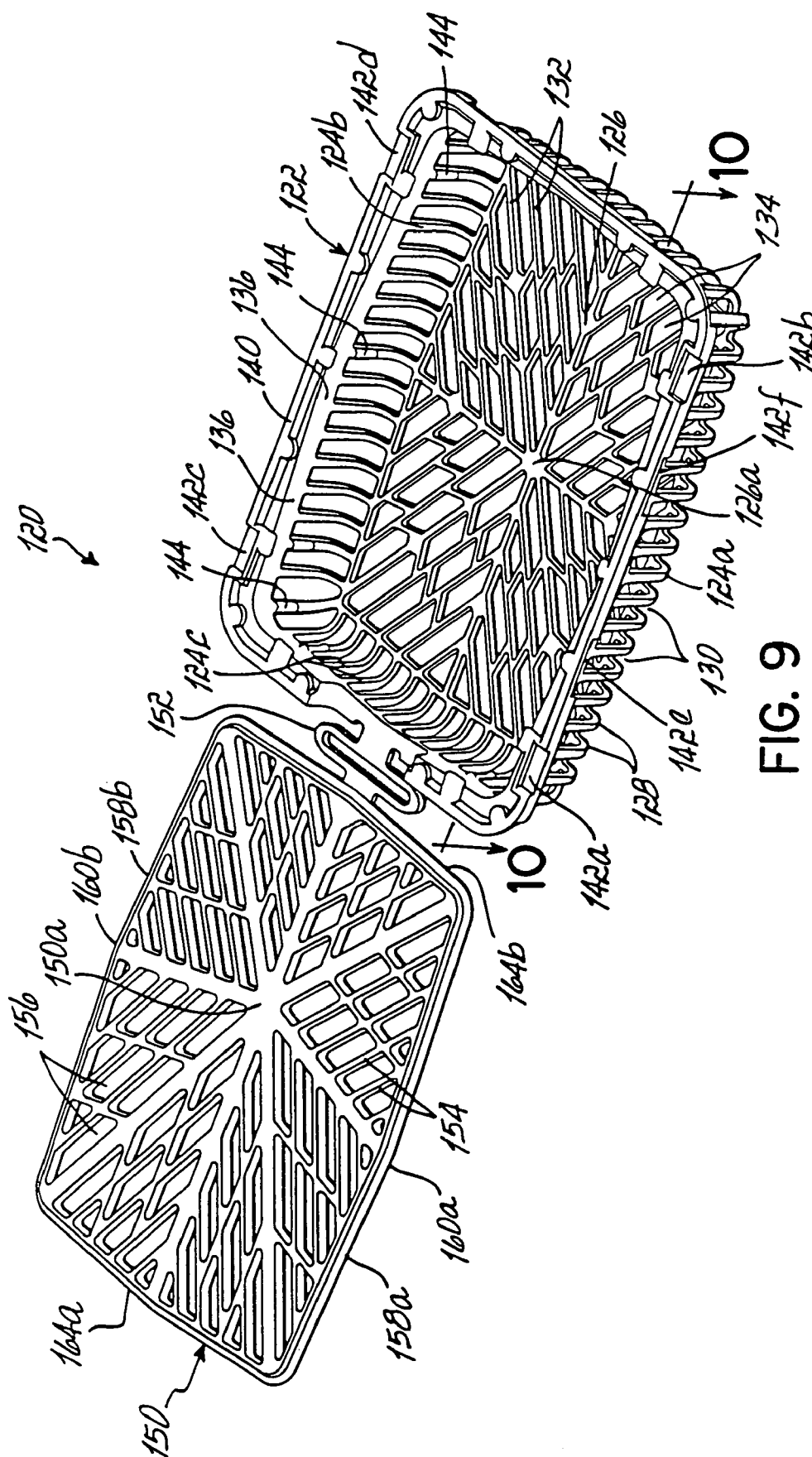
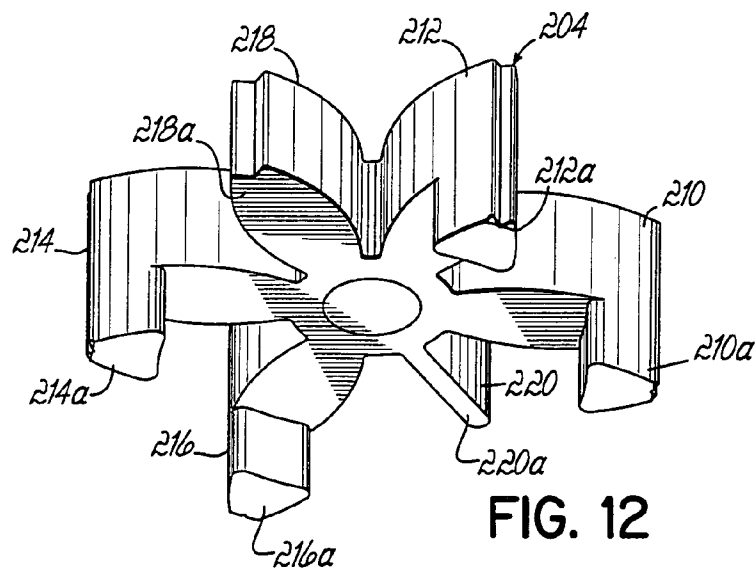
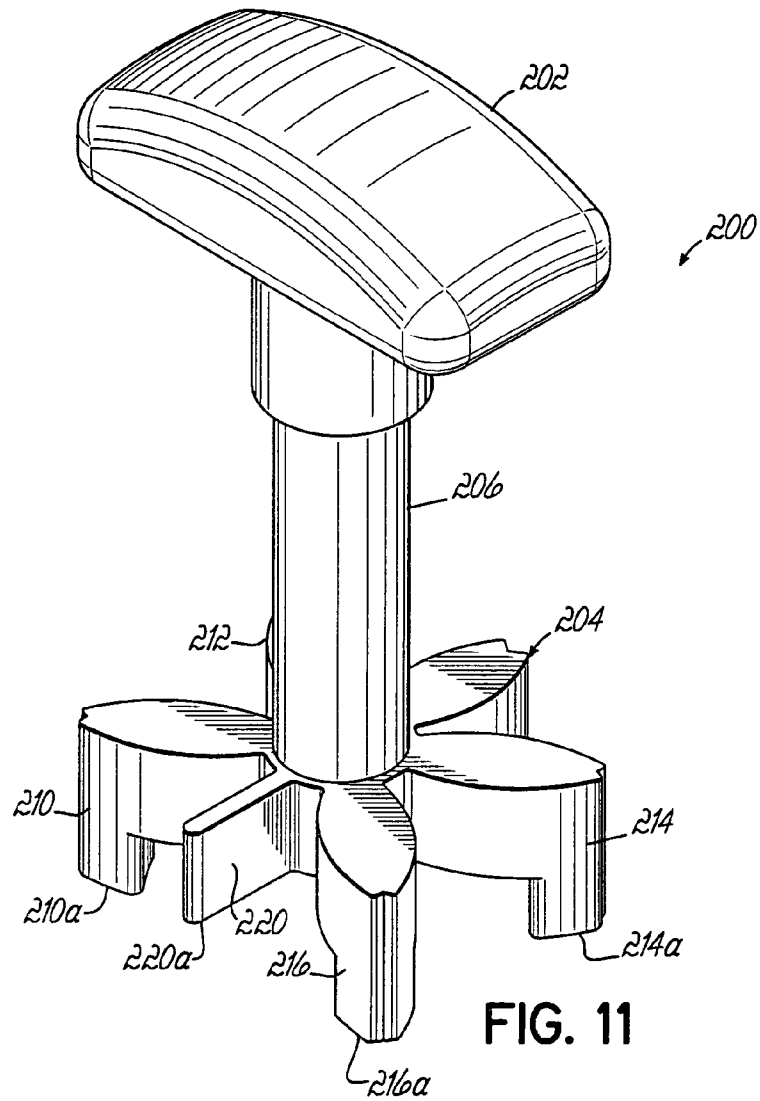


FIG. 9



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**CASSETTE AND EMBEDDING ASSEMBLY  
FOR HANDLING AND HOLDING TISSUE  
SAMPLES DURING PROCESSING,  
EMBEDDING AND MICROTOME  
PROCEDURES, STAGING DEVICES  
THEREFORE, AND METHODS THEREFOR**

The present application is a continuation of PCT Serial No. PCT/US02/30775 filed on Sep. 26, 2002 (now pending), the disclosure of which is hereby fully incorporated by reference herein.

**FIELD OF THE INVENTION**

The present invention generally relates to supports for handling and embedding tissue samples for pathological analysis and, more particularly, to cassettes which can receive one or more tissue samples and be embedded and subsequently microtomed with the tissue sample or samples.

**BACKGROUND OF THE INVENTION**

To accurately diagnose various tissue diseases and conditions, medical personnel must remove one or more samples of tissue from the body of a patient. This process of harvesting tissue from the body is known as a biopsy. Once the tissue sample or samples are removed and sent to a pathology laboratory, the tissue will go through a series of procedures performed by a histotechnician and, ultimately, a pathologist, in order to diagnose the tissue. The present invention generally relates to those procedures that are normally performed by the histotechnician to prepare the tissue sample or samples into slides that may be analyzed under a microscope by the pathologist.

Although the singular term "sample" is used throughout this specification, it should be understood that this term likewise encompasses plural "samples" as well. Once a tissue sample is removed from the body of a patient, it is typically placed into a specimen container containing a tissue fixative solution and then the container is transported to a pathology laboratory. The tissue will undergo a process known as "grossing-in" in the pathology lab during which a histotechnician will retrieve the tissue sample from the container, typically cut the tissue into appropriate sizes for tissue processing, place individual samples into the appropriate sized small plastic tissue cassettes, and assign tracking numbers to each cassette. These tracking numbers are then logged into a tracking system used in the laboratory. For the smallest tissue samples, which may only be scrapings, the cassette will have fine mesh openings on the sides and bottoms. In other situations involving very small tissue samples, the samples are placed into a bag that resembles a tea bag and prevents the smallest tissue samples from escaping. Larger tissue samples are placed into cassettes having somewhat larger slotted openings which are again smaller than the tissue sample inside the cassette.

The cassettes are then placed into a stainless steel perforated basket and run through a tissue processing machine, often overnight. This machine uses a combination of vacuum, heat, and chemicals to remove the interstitial fluids. Once the fluids have been removed from the tissue samples, the processing machine immerses the tissues samples in a bath of molten paraffin so that the interstices in the tissue are replaced with paraffin. The histotechnician then removes the basket from the machine and removes the individual tissue cassettes. At an embedding station, which has a molten paraffin reservoir and dispenser, the histotechnician will individually

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remove the tissue from each cassette. The histotechnician must carefully orient the tissue sample, based on tissue type, into a stainless steel base mold which is roughly the size of the tissue cassette and is partially filled with molten paraffin. The tissue sample must be manually held, typically using forceps, against the bottom of the mold. If it is not, this could compromise the ability to make proper slices of the tissue later in the microtome. The molten paraffin is then rapidly cooled on a refrigerated plate, which may be a thermal electric cooler (TEC), to partially solidify the paraffin thereby holding the tissue sample in the proper orientation against the bottom of the mold. The cassette is then placed on top of the base mold and paraffin is poured through the opened top of the cassette into the base mold. The cassette changes its function at this point in the procedure from a tissue holding component to a fixation device for later use in taking shavings or slices from the solidified paraffin in a microtome. The base mold is chilled until all of the molten paraffin has solidified and the histotechnician removes the stainless steel base mold from the block of embedded paraffin. The tissue sample is thus embedded within a rectangular block of paraffin with a plastic tissue cassette on the opposite side which will then be used as a holder in the chuck of the microtome. As with the tissue processing machine, the embedding process is accomplished in a batch fashion during which an average histotechnician may embed approximately 40 to 60 cassettes per hour.

The blocks of hardened paraffin containing the embedded tissue samples are then ready to be sliced into extremely thin sections for placement on a microscope slide. The histotechnician mounts the embedded tissue block in a chuck on the microtome which is sized to accept the side of the block that has the embedded plastic cassette. The histotechnician can then begin slicing the paraffin block which has the tissue sample embedded opposite to the plastic cassette surface. This yields a ribbon of individual slices of the tissue embedded in the paraffin. The action of the microtome causes the individual slices to stick together when done properly and, subsequently, these very thin ribbons of slices are floated into a water bath and a glass slide is carefully placed underneath the slice. The slice, with the thin sectioned tissue sample embedded therein, is then adhered to the top of the slide.

When the histotechnician has enough slides from the tissue sample, the slides are placed into an automatic staining machine. The staining machine goes through a series of infiltrating steps to stain the different tissue and cells of the slide different colors. This helps the pathologist identify different structures and makes it easier to find any abnormalities in the tissue. After the staining procedure is complete, the slides are cover slipped and prepared for the pathologist to place under a microscope to analyze.

Based on the summary of the procedure provided above, it will be appreciated that conventional tissue sample handling and processing is a very labor-intensive process involving several manual steps performed by a histotechnician. Thus, repetitive stress injuries such as carpal tunnel syndrome are prevalent. This is especially true with the tissue sample embedding process. These multiple manual operations and repeated tissue handling increase the likelihood of human error and, moreover, require highly trained and skilled histotechnicians to ensure that the tissue samples ultimately adhered to the slides for analysis by the pathologist are in an optimum condition and orientation to make accurate diagnoses.

U.S. Pat. No. 5,817,032 (the '032 patent) discloses various improvements to this area of technology, including new manners of holding tissue samples during the grossing in, embedding, and microtome or slicing procedures. More specifically,

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the '032 patent relates to a tissue trapping and supporting device, which may be a cassette, and which may be cut with a microtome. When a cassette is used, the tissue sample is immobilized within the cassette and subjected to the process for replacing tissue fluids with paraffin. Then, the tissue sample and the cassette are sliced at the same time for mounting on microscope slides. Because the tissue sample is never removed from the cassette from the time it is processed in the tissue processing machine to the time that it is cut with the microtome, a significant amount of handling time is saved. Moreover, the chance for human error or tissue loss due, for example, to dropping the tissue during handling, is significantly reduced due to the elimination of separate tissue handling steps. This patent also generally discusses an automated process which, in conjunction with the novel tissue cassettes, even further reduces the handling steps during the entire procedure.

In spite of the various advances made in this field, there is an increasing need for additional improvements related to increased production capability and increased and more consistent quality of embedded tissue samples and resulting slices or ribbons of embedded tissue which will be subject to diagnosis.

#### SUMMARY OF THE INVENTION

Generally the invention relates to a cassette for holding a tissue sample during an embedding and microtoming or slicing process. The cassette includes a body with a bottom wall and a plurality of side walls extending upwardly with respect to the bottom wall to define an interior space for receiving the tissue sample. The bottom wall and the plurality of side walls are constructed of a material capable of being sectioned in a microtome. Preferably, the cassette material is also resistant to any type of degradation during processing which would compromise its ability to function in accordance with the invention. In a first aspect of the invention, the plurality of side walls comprise first and second side walls on opposite sides of the bottom wall each including portions angling from approximate midpoints of the first and second side walls toward the other of the first and second side walls on the opposite side of the bottom wall. In the preferred embodiment, the two longest side walls of the four side walls comprising a rectangular cassette are generally V-shaped in a direction away from the interior of the cassette. This presents an apex of the V-shape to the microtome blade, after the embedding process is complete, which assists with the cutting action. Specifically, this feature has been found to reduce or prevent the hardened paraffin from breaking or cracking away from the cassette side wall material while making slices in the microtome.

The cassette preferably further includes a lid configured to be coupled with the body for movement between open and closed positions. The lid may be depressed downwardly on top of the tissue sample in the cassette interior. The lid is preferably stiffer than the bottom wall of the cassette. This feature allows the lid to position the tissue sample in the cassette parallel to the bottom of the mold during the embedding process. More specifically, the stiffer lid pushes the tissue sample and the more flexible bottom wall of the cassette against the rigid bottom of a base mold while the molten paraffin solidifies. This helps ensure that the bottom wall of the cassette can be removed in its entirety during a facing operation in the microtome prior to slicing the tissue sample, and that the tissue is positioned flatly against the bottom wall of the cassette.

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In another aspect of the invention, the side walls of the cassette are perforated such that there is at least a ratio of about 2.5:1 of open area to solid material area whereby the solidified paraffin occupies the open area of the side walls.

This ratio may be altered by using different materials for the cassette and/or paraffin embedding media. For instance, a higher molecular weight paraffin or lower moneluculer weight cassette will allow the ratio to vary somewhat. Currently, an industry standard paraffin (e.g., Sakura VIP processing/embedding medium) works best with a ratio of at least about 3.0:1 and, more preferably, at least about 3.5:1. This reduces the amount of cassette material that must be cut by the microtome blade while taking slices of the tissue and, therefore, increases blade life and quality of the resulting ribboned, embedded tissue samples. In addition this ratio assures that the paraffin is strong enough not to fracture when cut by the microtome blade. Another feature that results in similar advantages involves forming the side walls from ribs and offsetting the ribs of one of the first and second side walls with respect to those of the opposite side wall along the side wall length. Thus, the microtome blade will contact a more uniform amount of the cassette material along its length during each pass. This significantly reduces blade wear while cutting through the cassette material. Reduced blade wear is advantageous to keeping blade costs under control as the majority of blades used are disposable.

In another aspect of the invention, the cassette further includes a flange extending along upper portions of at least two of the side walls. The flange includes depressions configured to register with detents in a frame during the tissue embedding process. This increases the effective height dimension of the cassette interior, thereby allowing more tissue to be placed in the cassette and more passes to be made in the microtome. In this regard each pass of the microtome may only take a 5 micron slice. Therefore, using depressions having a depth, for example, of 0.14" can allow about 70 more slices to be taken in the microtome.

The invention further contemplates the various unique assemblies of two or more of the tissue cassette, frame, and base mold as disclosed herein. With respect to the frame and base mold, for example, structure is provided to physically hold the frame against the base mold. In the preferred embodiment, a seal is provided to perform this holding function and also to prevent leakage of liquid paraffin from the base mold.

In another aspect, the invention contemplates a device for staging a tissue sample cassette from an upper position in a frame used during a tissue embedding process to a lower position in the frame. The device includes a handle, a staging mechanism coupled with the handle and configured to engage an upper surface of the cassette, and a stop operatively coupled to the handle and configure to stop vertical movement of the staging mechanism when the cassette reaches the lower position in the frame. The staging mechanism further comprises a plurality of fingers which are configured to engage a corresponding plurality of locations on the upper surface of the cassette. For example, four fingers may be provided for engaging four corner locations on the cassette. This helps ensure that the cassette is engaged with at least four pairs of detents located proximate corner portions of the cassette thereby positioning the bottom wall of the cassette parallel to and against the bottom wall of the base mold.

In one embodiment, the staging device is a rigid member and the stop comprises a fixed stop member coupled for movement with the staging mechanism and configured to stop against an upper surface of the frame. In another embodiment, the device includes a stabilizing mechanism coupled with the

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handle and moveable relative to the staging mechanism. The stabilizing mechanism is configured to engage an upper surface of the frame as the staging mechanism moves the cassette from the upper position to the lower position within the frame. In this embodiment, the staging mechanism is normally spring-biased into an upward position and is forced downward against the spring bias when moving the cassette from the upper position to the lower position. The stop in this embodiment further comprises respective surfaces of the handle and stabilizing mechanism which engage one another when the staging mechanism has placed the cassette into the lower position. The staging devices of this invention ensure that the cassette is fully staged into the base mold, while ensuring that the cassette is not pushed too far through the frame. Moreover, the staging devices ensure that the bottom wall of the cassette and, therefore, the tissue sample, lay flat against the bottom of the base mold. This improves the efficiency and quality of tissue shavings later made in the micro-

tome. The invention also encompasses the various methods of using the tissue cassette and cassette/frame/base mold assemblies as disclosed herein.

These and other objects, advantages, and features of the invention will become more readily apparent to those of ordinary skill in the art upon review of the following detailed description taken in conjunction with the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an exploded perspective view of an assembly including the tissue cassette, the frame into which the cassette is inserted and the base mold into which the frame and cassette assembly are inserted.

FIG. 2 is an assembled perspective view of the tissue cassette, frame and base mold.

FIG. 3 is a cross sectional view taken along line 3-3 of FIG. 2 and showing the tissue cassette in its initial upper position.

FIG. 4 is an assembled perspective view, similar to FIG. 2 but illustrating the second, staged position of the tissue cassette.

FIG. 5 is a cross sectional view taken along line 5-5 of FIG. 4 and showing the tissue cassette staged down into its second, lower position against the bottom of the base mold.

FIG. 6 is a cross sectional view of the frame and the tissue cassette embedded in material such as paraffin, after release from the base mold.

FIG. 7 is a perspective view of a manual, spring-biased staging device.

FIG. 8 is a longitudinal cross sectional view of the staging device of FIG. 7 being used to stage a tissue cassette through the frame and into the base mold.

FIG. 9 is a perspective view of a tissue cassette constructed in accordance with a second embodiment of the invention.

FIG. 10 is a cross sectional view taken along line 10-10 of FIG. 9.

FIG. 11 is a perspective view of a manual staging device constructed in accordance with a second embodiment.

FIG. 12 is a bottom perspective view of the staging mechanism associated with the staging device of FIG. 11.

#### DETAILED DESCRIPTION

Turning first to FIGS. 1-3, a tissue cassette 10 constructed in accordance with the invention is received within a frame 12 and the tissue cassette 10 and frame 12 are then positioned in a base mold 14. After a staging operation the base mold 14 is

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subsequently filled with liquid paraffin, as described further below. Tissue cassette 10 includes a perforated body 20 formed by four side walls 22a, 22b, 22c, 22d and a bottom wall 24. Each wall is preferably constructed with perforations or openings 26 and ribs 28. An upper flange 30 surrounds and extends outwardly from the respective side walls 22a-d. A lid 32 is attached to body 20 by a hinge 34 which allows vertical movement of lid 32 into the interior of body 20 for retaining one or more tissue samples against bottom wall 24. Lid 32 is likewise formed with perforations 36 which may be elongated and generally separated by ribs 38. As shown best in FIG. 1, elongated perforations 26, 36 on the bottom wall 24 and lid 32 respectively extend toward central areas 24a, 32a of the bottom wall 24 and lid 32. This assists with allowing a mold (not shown) to fill with material, such as PFA, during the molding process of cassette 10.

Lid 32 is formed with a shape along its circumference which compliments the shape of side walls 22a-d. In this regard, respective lengthwise side edges 40a, 40b of lid 32 are formed in the shape of a shallow "V" and each includes an apex 42a, 42b generally at the central portion thereof. This likewise corresponds to the lengthwise shallow "V" shape of side wall 22a and opposite side wall 22c. Thus, when the embedded tissue cassette is later placed in a microtome and sections are sliced from the embedded tissue cassette, the microtome blade will initially contact the apex corresponding of side wall 22a or 22c, depending on which side is facing up in the microtome. This has been found to increase the quality of ribboned slices being made from the embedded tissue cassette. That is, little or no fracturing of paraffin will occur at the paraffin/cassette interfaces.

Frame 12 more specifically includes an open interior 50 which receives cassette 10, and an angled front wall 52 which may be used for recording indicia, such as patient data. Respective upper and lower sets of detents 54, 56 and 58, 60 extend inwardly into the open interior 50 of frame 12. Initially, tissue cassette 10 is held between the upper pairs of detents 54, 56 by pressing the upper flange 30 downwardly past the upper sets of detents 54 and against the lower set of detents 56 after one or more tissue samples have been placed into cassette body 20 and lid 32 has been closed. Respective detents 61 extend inwardly from side walls 22a-d to allow lid 32 to be snapped and retained in a closed position. During a staging operation, tissue cassette 10 will be moved vertically downwardly through frame 12 until flange 30 snaps past detents 58 and rests against detents 60 at a lower position (FIG. 5). Recesses 62, 64 are formed in at least two opposite side walls for allowing fingers of a gripper mechanism to register with the assembly during automated handling and embedding operations, as necessary. Preferably side walls 66a, 66b contain these recesses 62, 64, while additional side walls 66c, 66d may include additional structure allowing automated handling, or allowing other functions as necessary. Base mold 14 includes an open interior 70 for receiving frame 12 and may be surrounded by a resilient elastomeric seal 72 which prevents the escape of liquid paraffin during the embedding process. This eliminates an additional step of scraping off excess hardened paraffin from frame 12 after the embedding process is complete. Such excess paraffin can prevent the frame from properly fitting in a microtome chuck.

By comparing FIGS. 2 and 3 to FIGS. 4 and 5, it will be appreciated that during the staging process, tissue cassette 10 is moved vertically downward into the interior 70 of base mold 14 such that the bottom 24 of tissue cassette 10 contacts the bottom 74 of base mold 14. At this position, flange 30 of tissue cassette body 20 is received between respective detents 58, 60 holding it in this lower position. Frame 12 is preferably

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press fit and held frictionally within base mold **14** by one or more resilient seals **72**. Seal **72** performs two functions in this embodiment. First, it physically holds frame **12** within base mold **14** by friction. This prevents the frame **12** and attached cassette **10** from floating or otherwise moving while base mold **14** is filled with paraffin. Second, it prevents the liquid paraffin from escaping from base mold **14** in the area between outer walls **12a** of frame **12** and interior **70** of base mold **14**. It will be appreciated that a holding member other than seal **72** may be used instead to physically hold frame **12** against base mold **14**. In this case, seal **72** may not be necessary. As some examples, such holding members may be clamps, fasteners, spring members or weights. In these figures, the tissue sample or samples within cassette **10** have been deleted for clarity. However, it should be understood, that lid **32** will be depressed down onto one or more tissue samples **80** contained with the interior of cassette body **20** (FIG. 6). While the assembly of cassette **10**, frame **12** and base mold **14** is in the staged configuration shown in FIGS. 4 and 5, liquid paraffin is introduced through the open interior of frame **12** and through the perforations **26**, **36** of cassette **10** into the interior **70** of base mold **14**. The liquid paraffin is then preferably cooled on a suitable cooling device, such as a TEC, and the assembly of the tissue cassette **10**, frame **12**, embedded tissue sample **80** and hardened paraffin **82** is removed from base mold **14** as shown in FIG. 6. Frame **12** may then be used as a device to fix the assembly in a microtome chuck and slices are then taken from the bottom surface **82a** of paraffin **82**. Initially, a facing blade is used to remove the initial layer of paraffin **82** and bottom wall **24** of tissue cassette **10**. At this point, a different microtome blade may be used to take ribboned slices or shavings of tissue sample **80** and the surrounding paraffin **82** and side walls **22a-d** of tissue cassette body **20**. Often times the same blade that is used for facing may also be used for sectioning.

FIGS. 7 and 8 illustrate one type of staging device **100** which may be used in conjunction with the tissue cassette **10**, frame **12** and base mold **14** described above. Staging device **100** includes a handle **102** at an upper end and a staging mechanism **104** at a lower end. Staging mechanism **104** is coupled to a hollow cylinder **106** extending upwardly into a shroud **108** rigidly connected with handle **102**. A plurality of four stabilizer members **110** are rigidly coupled with cylinder **106**, while a plurality of preferably movable staging fingers **112** are coupled to a reciprocating shaft **114** which extends through cylinder **106**. Shaft **114** is rigidly connected to handle **102** and is biased therewith in an upward position by a spring **116** positioned between a bottom surface **102a** of handle **102** and an upper surface **106a** of cylinder **106**. Thus, it will be appreciated that stabilizer members **110** may be placed against an upper side of frame **12** for stabilizing purposes and handle **102** may then be depressed downwardly as indicated by arrow **118**. This moves reciprocating shaft **114** and the attached staging fingers **112** downwardly against tissue cassette **10** thereby moving the tissue cassette **10** relative to frame **12** from its upper position shown in FIG. 3 to its lower, staged position shown in FIG. 5. To avoid pushing cassette **100** too far through frame **12**, surface **108a** stops against surface **110a** as flange **30** reaches its lower position between detents **58**, **60**.

FIGS. 9 and 10 illustrate a second embodiment of a tissue cassette **120**. Tissue cassette **120** includes a cassette body **122** having four side walls **124a-d** surrounding an open interior and bounded on a bottom side by a bottom wall **126**. Side walls **124a-d** are constructed of ribs **128** separated by perforations **130** and bottom wall **126** is constructed by ribs **132** separated by perforations **134**. Ribs **128** of side wall **124a** are offset in a lengthwise direction relative to ribs **128** of opposite side wall **124b**, as shown by distance "d" in FIG. 10. Distance "d" may vary, however, in this embodiment, on average, it is approximately 0.015" to 0.030". Offsetting ribs **128** in this manner ensures that a microtome blade passing through walls **124a** and **124b** contacts a more uniform amount of cassette material along its length. This leads to longer blade life, more uniform blade wear and more consistent high quality slices of embedded tissue. Ribs **132** and perforations **134** extend in their lengthwise direction toward a central area **126a** of bottom wall **126**. In this preferred embodiment, side walls **124a-d** are constructed such that the ratio of plastic cassette material, such as PFA, to the open area formed by perforations **130** is approximately 3.7:1. To achieve at least this ratio in the preferred embodiment, ribs **128** have a width W1 of approximately 0.010" to 0.014" while perforations **130** have a width W2 of approximately 0.040" to 0.050". This perforated area, which is below solid side wall portion **136** is the area which will be cut with a microtome blade after embedding of tissue sample **80** (FIG. 6). Especially when using PFA with a Shore D hardness of 48 to 55 as the material for cassette **120**, in combination with the industry standard paraffin embedding material mentioned above, this ratio of cassette material to open area has been found to improve the quality of cuts made through the embedded cassette and increase blade life significantly. Depending on the respective molecular weights of the embedding material and the cassette material, this ratio may be increased or reduced.

A flange **140** surrounds the upper side of cassette body **122** and contains respective upwardly facing depressions **142a**, **142b**, **142c**, **142d**. A number of downwardly facing depressions **142e**, **142f** (only two of several referenced) are also contained in flange **140**. These depressions respectively register with at least detents **54**, **56** and **58**, **60** of frame **12** (see FIGS. 3 and 5). It will be appreciated that additional lower detents may be formed on frame **12** to provide further support and prevent cassette **10** from being pushed too far through frame **12**. In such a case, additional depressions are formed in the underside of flange **140** to accommodate the additional supportive detents. The depressions allow the effective outward extension of cassette body **120** from frame **12** to be increased thereby increasing the number of slices that may be taken from the embedded cassette in a microtome. This can be important in many situations in order to obtain the desired slides for pathologic examination.

Detents **144** are also formed on side walls **124a-d** for retaining a lid **150** in place. Lid **150** is coupled to cassette body **122** by a hinge **152**. Lid **150** is formed to be stiffer than bottom wall **126** of cassette body **122** so that lid **150** may be used to press the tissue sample against the bottom wall **126** and to press the bottom wall **126** against the rigid bottom **74** of base mold **14** (FIG. 5). Lid **150** is formed by a plurality of ribs **154** generally separated by perforations **156** and each extending in a lengthwise elongated fashion toward a central area **150a** of lid **150**. Lid **150** further includes side edges **158a**, **158b** extending lengthwise thereon and having a shallow "V" shape with a central apex **160a**, **160b**. Angle  $\alpha$  (FIG. 10) is preferably about 4°. As with the first embodiment, the shape of side edges **158a**, **158b** complements similar shape of side walls **124a**, **124b** of cassette body **122**. In this regard, these side walls **124a**, **124b**, either one of which may be the initial wall cut in the microtome, include respective apices **162a**, **162b**. Respective ends **164a**, **164b** of lid **150** also

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complement the similar shape of cassette body side walls **124c**, **124d** to achieve a close fit of lid **150** within cassette body **122**. The use of cassette **120** in the process of embedding one or more tissue samples, including the staging process within frame **12** and the embedding or molding process within base mold **14** is the same as described above with regard to FIGS. 1-8.

FIGS. **11** and **12** illustrate a manual staging device **200** in accordance with a second embodiment. Staging device **200** comprises an upper handle **202** and a lower staging mechanism **204** coupled together rigidly by a shaft **206**. In FIG. **12**, handle **202** and shaft **206** have been deleted for clarity. Staging mechanism **204** preferably comprises a plurality of four staging members **210**, **212**, **214**, **216** extending generally radially outward from shaft **206** and each including a finger portion **210a**, **212a**, **214a**, **216a**. The finger portions **210a**-**216a** respectively engage upper corner portions of cassette **10** (FIG. **1**) or cassette **120** (FIG. **9**). When the user then pushes downward on handle **202**, finger portions **210a**-**216a** urge cassette **10** or cassette **120** downward into the lower, staged position (FIG. **5**). To prevent the cassette from being moved too far downward, a pair of stops **218**, **220** are provided on staging mechanism **204**. In this embodiment, stop surfaces **218a**, **220a** stop against the upper surface of frame **12** as cassette **10** or cassette **120** reaches the lower, staged position (FIG. **5**). Thus, the distance between the lower ends of finger portions **210a**-**216a** and stop surfaces **218a**, **220a** equates to the distance between upper detent pair **54**, **56** and lower detent pair **58**, **60**. It will be appreciated that other forms of staging mechanisms and stops may be provided while falling within the spirit and scope of the invention.

While the present invention has been illustrated by the description of the various embodiments thereof, and while the embodiments have been described in considerable detail, it is not intended to restrict or in any way limit the scope of the appended claims to such detail. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and methods and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the scope or spirit of Applicant's general inventive concept.

What is claimed is:

**1.** A cassette for holding a tissue sample, comprising:  
a body including a perforated bottom wall and a plurality of perforated side walls extending upwardly with respect to said bottom wall to define an interior space for receiving the tissue sample;

said bottom wall and said plurality of side walls being constructed of a material capable of being successfully sectioned in a microtome and resistant to degradation during histologic tissue processing, wherein said plurality of side walls comprise first and second side walls each having a length and extending upwardly from opposite edges of said bottom wall, at least one of said first and second side walls including portions angling toward the other of the first and second side walls on the opposite edge of the bottom wall to define an outwardly projecting portion proximate a midpoint along said length of said at least one side wall.

**2.** The cassette of claim **1**, further comprising a lid configured to be coupled with said body and movable between open and closed positions, said lid being stiffer than said bottom wall.

**3.** The cassette of claim **1**, wherein said plurality of side walls comprise perforated walls in which the ratio of open area to solid area is at least about 3.5:1.

**4.** The cassette of claim **1**, wherein said plurality of side walls comprise perforated walls in which the ratio of open area to solid area is at least about 3.0:1.

**5.** The cassette of claim **1**, wherein said plurality of side walls comprise perforated walls in which the ratio of open area to solid area is at least about 2.5:1.

**6.** The cassette of claim **1**, wherein said first and second side walls include a length and comprise perforated walls constructed with spaced apart ribs, said ribs of said first side wall being offset along said length with respect to said ribs of said second side wall.

**7.** The cassette of claim **1**, further comprising:

a flange extending along upper portions of at least two of said side walls, said flange including depressions configured to register with detents in a frame during a tissue embedding process.

**8.** The cassette of claim **1**, wherein said bottom wall includes a central area and comprises a perforated wall constructed with spaced apart ribs, said ribs angling toward said central area.

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